

# ✱Effect of Propionic Acid on Aflatoxin Levels of Stored Georgia 1981 Dent Corn Samples<sup>1</sup>

O.L. SHOTWELL, G.A. BENNETT, W.F. KWOLEK<sup>2</sup> and C.W. HESSELTINE, Northern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, Peoria, IL 61604

Purchased by U. S. Dept. of Agriculture for Official Use

## ABSTRACT

In 1981, 75 corn samples (ca. 60 ears per sample) were collected from 4 areas in Georgia to study methods for handling freshly harvested samples. The collection was not representative of the Georgia corn crop. Every sixth ear was husked and dried the day of collection. The rest of the corn was shipped, without husking, to Peoria in cardboard boxes. When the remaining undried, unhusked samples arrived in Peoria, they were husked and randomly separated into 5 equivalent samples. One set of 75 samples was shelled and dried as soon as possible to avoid further aflatoxin formation; another set of 75 samples was stored for 6 weeks before shelling and drying. The remaining 3 sets were placed in plastic bags with 5%, 2% and 1% Monoprop (1 part propionic acid plus 1 part Versite) and stored for 6 weeks before shelling and drying. The samples dried in Georgia had an average total aflatoxin level of 199 ng/g. Samples shelled and dried immediately after arriving in Peoria had an average level of 640 ng/g. Samples shelled and dried after a 6-week storage period had average aflatoxin levels of 601 ng/g. Samples stored 6 weeks with 5%, 2% and 1% Monoprop (2.5%, 1% and 0.5% propionic acid) had average aflatoxin levels of 340 ng/g, 351 ng/g and 376 ng/g, respectively. Monoprop cannot be used to maintain the integrity of high-moisture corn samples collected for aflatoxin analysis.

## INTRODUCTION

Aflatoxin levels can increase in field-contaminated corn if the corn is stored after harvest at high moisture. One report indicated a ca. 10-fold increase in aflatoxin levels (from 200 to 2,300 ng/g), in a 3-day period after harvest, in high-moisture corn that was not dried (1). In 1979, freshly harvested dent corn was collected in Georgia at maturity and separated into two sets of 57 matched samples (2). One set was dried in Georgia the day of collection; the other set was shipped to Peoria (NRRC) before drying. The mean total aflatoxin level in the samples dried in Georgia was 36 ng/g; the mean level in the matched samples shipped to Peoria before drying was 78 ng/g. Aflatoxin formed during shipment of the freshly harvested undried corn from Georgia. Volatile organic acids, such as acetic or propionic acids, have been suggested to prevent mold growth and aflatoxin formation in corn samples stored at high moisture (1). However, when we attempted to prevent mold growth and aflatoxin formation during storage of undried corn samples in plastic bags with 5% Monoprop, aflatoxin levels decreased (3). Monoprop is a mixture of 1 part propionic acid plus 1 part Versite. Versite, a horticultural vermiculite, serves as an inert support facilitating the handling of propionic acid in the field.

We tried the storage of freshly harvested 1981 corn samples in the presence of lower levels of Monoprop—1% and 2%—as well as 5% to see if low levels could be used to prevent aflatoxin formation after collection. We are now reporting the results of that study.

## MATERIALS AND METHODS

### Sample Collection and Handling

Samples of 1981 mature dent ear corn (about 60 ears per location) were harvested from July 29 to September 7 in

each of 75 locations in the coastal plain region of Georgia. One set of subsamples consisted of every sixth ear of corn from a lot of about 60 ears that was husked and placed in a forced-air oven at 60 C on the day of collection. The moisture of the samples was not determined. Drying took 72 hr, after which the samples were shipped to NRRC in Peoria to be shelled, prepared for analysis and analyzed for aflatoxin. The remaining unhusked ears of corn, which had not been dried, were placed in corrugated cardboard boxes for shipment to NRRC. Samples were in transit for an average of 7 days. The average temperature to be expected in the areas through which the samples were shipped is 74 F. In these areas, over the years, the average daily maximum temperature has been 84 F; the average daily minimum temperature has been 64 F. On arrival in Peoria, the husks were removed from the ears and the 75 samples were separated into 5 sets of matched subsamples. One of the 5 subsamples was shelled and dried as soon as possible (overnight at 80 C). The other 4 sets of subsamples were stored for 6 weeks at 75-85 F: one was stored untreated in paper sacks before shelling and drying. The remaining 3 sets of subsamples were placed in airtight plastic bags with 3 different levels of Monoprop—5% (5% of the weight of the corn or 2.5% propionic acid), 2% and 1%. At the end of 6 weeks, the untreated subsamples and the 3 subsamples treated with 5%, 2% and 1% Monoprop were shelled and dried. Samples were dried to less than 14% moisture.

### Preparation of Samples for Analysis

Each subsample of shelled, dried corn was coarsely ground in a Straub disc mill. The coarsely ground corn was finely ground to pass through a No. 20 sieve in a 6-in. Raymond hammer mill fitted with a screen with  $\frac{1}{8}$ -in. perforations before being blended in a Hobart planetary mixer for 15 min.

### Analysis for Aflatoxin

Subsamples (50 g, ground and blended) were assayed for aflatoxins by the method designated as the CB (Contaminants Branch) method approved for corn by both the Association of Official Analytical Chemists (4) and the American Association of Cereal Chemists (5). Selected samples that had large decreases in aflatoxin levels after treatment and storage with 1%, 2% or 5% Monoprop were analyzed by the method approved by the AOAC for cottonseed (6), the method developed for aflatoxin in corn using thin-layer chromatography (TLC) or high-performance liquid chromatography (HPLC) (7) and a method for aflatoxin in mixed feeds (8).

## RESULTS AND DISCUSSION

Aflatoxin incidences and levels in the 6 subsamples—the set dried in Georgia, the set dried in Peoria after shipping, the set stored for 6 weeks, and the 3 sets stored for 6 weeks with varying amounts of Monoprop—are summarized in Table I. The samples collected were not representative of the 1981 corn harvested in Georgia or any one area of Georgia. Samples were collected for this study from farms within 140 miles of Tifton, Georgia. Four areas were represented (Table II). Group 1 and 2 samples were collected

<sup>1</sup> Presented at the 75th AOCS meeting, Chicago, May, 1983.

<sup>2</sup> North Central Region, Agricultural Research Service, stationed at the Northern Regional Research Center, Peoria, IL 61604.

## EFFECT OF ACID ON AFLATOXIN IN CORN

TABLE I

Comparisons of Aflatoxin Levels in 1981 Corn Samples Dried in Georgia and Peoria After Shipping (in Husks), After Storage or Treatment with Monoprop<sup>a</sup>

Total aflatoxin (ng/g)	Dried in Georgia		Shipped to NRRC in husks before drying		Ears with husks removed stored 6 weeks before drying							
					Treated with Monoprop							
	No. of samples	%	No. of samples	%	Untreated	1%	2%	5%	No. of samples	%	No. of samples	%
ND <sup>b</sup>	11	14.7	11	14.7	11	14.7	12	16.0	11	14.7	9	12.0
<20	13	17.3	9	12.0	3	4.0	9	12.0	5	6.7	8	10.7
20-99	16	21.3	13	17.3	14	18.7	13	17.3	19	25.3	22	29.3
100-499	21	28.0	16	21.3	22	29.3	24	32.0	25	33.3	18	24.0
≥500	14	18.7	26	34.7	25	33.3	17	22.7	15	20.0	18	24.0
% Incidence	85.3		85.3		85.3	84.0		85.3		88.0		
% ≥20	68.0		73.3		81.3	72.0		78.6		77.3		
% ≥100	46.7		56.0		62.6	54.7		53.3		48.0		
Average level (ng/g), all samples	199		640		601	376		351		340		
Average level (ng/g), positive samples	233		750		697	448		410		388		

<sup>a</sup>Six sets of 75 matched samples.

<sup>b</sup>ND = Nondetected.

TABLE II

Total Aflatoxin in Samples of 1981 Georgia Dent Corn

Group	Number of samples	Total aflatoxin	
		Mean	Range
1	15	222.1	8-694
2	25	431.0	19-1112
3	23	37.6	0-128
4	12	5.4	0-61

near Tifton. Samples for group 3 came from a location southwest of Tifton, and those for group 4 came from a location northwest of Tifton. The samples collected further north in the 1981 collection were practically free of aflatoxin. The set of samples that was dried in Georgia best represents aflatoxin occurrence at harvest in the corn used in this study. The incidence of samples with detectable aflatoxin was 85.3%; the incidence of samples with aflatoxin at levels equal to or greater than 20 ng/g (the Food and Drug Administration guideline) was 68%. The average total aflatoxin level in samples collected and dried immediately for this study was 199 ng/g.

In 1981, the undried corn samples were not husked in Georgia before being shipped to NRRC. When the samples arrived in Peoria, all husks were removed immediately. A comparison of the results of aflatoxin analysis of subsamples dried in Georgia before shipping and those obtained of subsamples dried in Peoria on arrival revealed no difference in the incidence of nondetectable aflatoxin (Table I). However, the mean level of aflatoxin (199 ng/g) of the samples dried in Georgia the day of collection was significantly lower than the mean level of 640 ng/g in the set dried on arrival in Peoria. The incidence of aflatoxin levels of equal to or greater than 500 ng/g was 18.7% in the samples dried in Georgia and 34.7% in samples dried in Peoria. The husks probably prevented moisture from escaping; thus little drying could take place during the ca. 7 days in transit. In 1980, when husks were removed from all corn samples immediately after collection, no difference was found in mean levels of aflatoxin between samples dried in Georgia immediately after collection and those shipped without

husks and dried on arriving in Peoria (3). We recommend that husks be removed from corn samples at time of collection for aflatoxin analysis.

The mean aflatoxin levels in the untreated husked corn samples stored for 6 weeks did not increase (Table I). No significant difference was found between the mean toxin levels in corn samples dried in Peoria immediately after arrival (640 ng/g) and those stored in Peoria for 6 weeks before drying (601 ng/g). The samples treated with 1%, 2% and 5% Monoprop had significantly lower mean levels of aflatoxin—376, 351 and 340 ng/g—than the untreated samples stored for 6 weeks, and significantly higher levels than the samples dried in Georgia. Although the mean level of aflatoxin appeared to decrease as the percentage of Monoprop increased, the differences in the mean levels were not statistically significant. Two sample means, whose ratio exceeds the least significant ratio (for these samples, LSR = 1.43), differ significantly at the .05 level. The change in aflatoxin levels between the untreated samples and treated samples stored for 6 weeks was most noticeable in the incidence of samples containing toxin at levels equal to or greater than 500 ng/g. Of the untreated samples stored for 6 weeks, 33.3% had levels equal to or greater than 500 ng/g. The samples stored for 6 weeks with Monoprop had a 20-24% incidence of samples with levels equal to or greater than 500 ng/g.

No reports were found in the literature of aflatoxin destruction in corn caused by propionic acid. The hemiacetal or water adduct of aflatoxin B<sub>1</sub>, B<sub>2</sub>a, does form slowly in acidic aqueous or acetone solutions (9,10). No evidence of the presence of aflatoxin B<sub>2</sub>a in extracts of corn obtained by the CB method (4,5) was discovered. We analyzed selected samples, in which aflatoxin decreased markedly during the 6-week storage period in the presence of Monoprop, by 3 other methods using more polar extracting solvents. The 3 methods and the solvents for extraction were the official method adopted for aflatoxin in cottonseed (solvent, acetone/water [85:15]) (6), a method developed for corn (solvent, methanol/water [80:20]) (7) and a method developed for aflatoxin B<sub>1</sub> in mixed feeds (solvent, dichloromethane/20% citric acid [20:1]) (8). Aflatoxin B<sub>2</sub>a was not detected in the partially purified extracts obtained by any of the 3 methods. If B<sub>2</sub>a was formed, it

may have formed a complex with proteins in corn, causing it to be insoluble in solvents used for extraction.

#### ACKNOWLEDGMENT

L. M. Elam, J. Greer and M. S. Milburn prepared samples and made analyses.

#### REFERENCES

1. Wilson, D.M., W.W. McMillian and N.W. Widstrom, JAOCS 56:798 (1979).
2. Shotwell, O.L., W.F. Kwolek and C.W. Hesseltine, Ibid. 58: 980A (1981).
3. Shotwell, O.L., G.A. Bennett, W.F. Kwolek and C.W. Hesseltine, J. Assoc. Off. Anal. Chem. 66:204 (1983).
4. Official Methods of Analysis of the Assoc. Off. Anal. Chem., 13th Ed., AOAC, Washington, DC, 1980, Sec. 26.049, 26.026-26.031.
5. Methods of American Association of Cereal Chemists, 1972 Rev. to AACC Approved Methods 45-05:1-9, AACC, Minneapolis, MN.
6. Mycotoxin Separate from Chapter 26, Official Methods of Analysis of the Assoc. Off. Anal. Chem., 13th Ed., AOAC, Washington, DC, 1980, Sec. 26.A01-26.A07.
7. Thean, J.E., D.R. Lorenz, D.M. Wilson, K. Rodgers and R.C. Gueldner, J. Assoc. Off. Anal. Chem. 63:631 (1980).
8. Shannon, G.M., O.L. Shotwell and W.F. Kwolek, Method for Determining Aflatoxin B<sub>1</sub> in Mixed Feeds, Ibid. 66:224 (1983).
9. Pohland, A.E., M.E. Cushmac and P.J. Andrellas, Ibid. 51:907 (1968).
10. Pons, W.A., Jr., A.L. Cucullu, L.S. Lee, H.J. Janssen and L.A. Goldblatt, JAOCS 49:124 (1972).

[Received September 19, 1983]

---